

# Does melatonin as an irrigation solution increase the adhesive quality of root canal sealer?

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## Abstract

**Aim:** To evaluate the push-out bond strength of AH-Plus when Melatonin used as an endodontic irrigation.

**Material and Methods:** Ninety extracted human anterior incisors were selected randomly. The teeth were instrumented with R25 Reciproc® files and irrigated according to one of the irrigation protocols included as (n=15): G1: 5.25% NaOCl; G2: 17% EDTA; G3: 0.2% Melatonin; G4: 5.25% NaOCl + 17% EDTA; G5: 5.25% NaOCl + 0.2% Melatonin; G6: 0.2% Melatonin + 17% EDTA. After the root canal obturation procedures, roots were sectioned in coronal, middle and apical of root thirds. Push-out tests were performed Result were analyzed by ANOVA and Tukey tests (P<0.05).

**Results:** In the coronal thirds, there were significant differences among the groups. Melatonin+EDTA group showed had greater the bond strength than other groups, except NaOCl+Melatonin. In the middle thirds, NaOCl group showed significantly the lowest bond strength than other groups. In the apical thirds, NaOCl and melatonin groups showed significantly worse (P<0.05).

**Conclusion:** The use of Melatonin as a final irrigant significantly increased the push out bond strength of an AH-Plus sealer to root dentin. Further studies are needed to determine appropriate concentration and time used for melatonin.

**Keywords:** Melatonin; epoxy resin sealer; push-out bond strength.

## INTRODUCTION

Satisfactory root fillings had been defined as 'adequate seal' and 'absence of voids' (1). In root canal treatments, hermetically filled canals are required. Gutta-percha does not adhere to root canals. Adequate adhesion can be achieved in combination with root canal sealers. In previous studies, the adhesive properties of endodontic sealers have been reported to be important (2,3). These studies have stated that the resistance the displacement of a sealer may reduce leakage (4,5). It is reported that this chemical bonding to the root of the tooth increases the adhesion strength of the sealants to the root canal walls (6). Many factors can interfere with the adhesion of sealers to canal surface. Following instrumentation, smear layer is formed on root canal walls. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are the most widely used endodontic irrigation products (7,8). Nikaido et al.(9) reported that irrigation solution remnants, such as hydrogen peroxide or NaOCl, have unfavorable effects on the polymerization of the adhesive system, which leads to reduced adhesion. Chemical irrigation solutions are known

to cause changes of collagen of the root dentine and this affects the bond strength of the root canal sealers (10,11). The use of irrigation solutions such as EDTA with the capacity to demineralize the dentin surfaces may cause changes in the chemical and structural composition of the dentin, and hence may affect the bonding of the sealer to the dentin (12).

Melatonin was discovered about 60 years ago and is a hormone released from the pineal gland, whose importance has become more apparent in recent years. It is an excellent antioxidant because of its high diffusion ability and lipophilic structure; It is more efficient than other known antioxidants (13). There are also studies in the field of dentistry (14,15). Melatonin has also antimicrobial properties against various bacteria and viruses (16-18). Based on these properties, we speculate that Melatonin has the potential to increase the bond strength and to have potential to replace NaOCl as a root canal irrigation solutions.

This study was designed to examine the effect of root

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canal irrigation agents using 5.25% NaOCl, 17% EDTA and 0.2 % melatonin on push-out bond strength of an AH-Plus sealer to root dentine. The null hypothesis tested was that the investigated irrigation solutions would be no effect on the push-out bond strength of root canal filling.

## MATERIAL and METHODS

### Preparation of Solution

Melatonin solution used in our study was prepared by dissolving 0.2 g of melatonin (Aldrich Chemistry, St. Louis, USA) in 100 ml of 0.01% saline-ethanol solution to be 0.2% (w / v).

### Irrigation Procedure and Obturation

After the approval of the ethics committee (2018 / 04-13) ninety extracted human mature maxillary central incisor teeth with a single root and without root resorption was selected. The crowns were removed and a 13-mm root were obtained. Working length determination was achieved under stereomicroscope (Leica MZ 12.5, Heerbrugg, Germany) optical magnification (x25) by inserting a size 10 K file until it was visible at the apex and subtracting 1 mm from that length. Then, the root canals were prepared with R25 Reciproc® files (VDW GmbH, Munich, Germany) attached to an endodontic motor (VDW Reciproc Gold, Munich, Germany) using reciprocal mode under irrigation with following the irrigation protocols and the needle tip of the irrigating syringe positioned at 3 mm away from WL with the passive irrigation modality. For irrigation protocols all roots were distributed randomly into six groups of 15 roots each: G1:5.25% NaOCl (3 min); G2: 17% EDTA; G3: 0.2% melatonin (3 min); G4: 5.25% NaOCl + 17% EDTA (3 min); G5: 5.25% NaOCl + 0.2% Melatonin (3 min) ; G6: 0.2% Melatonin + 17% EDTA (3 min). Following irrigation procedure, Root canals dried with paper points and were obturated using AH-Plus (Dentsply, Konstanz, Germany) and gutta-percha by means of the cold lateral condensation technique. All specimens were stored in 100% humidity at 37 °C for 7 days to provide complete setting of AH-Plus.

### Push-Out Test

Similar to our previous study (5), after root filled, horizontal slices (1-mm-thick) were obtained from the coronal, middle and apical one thirds of the roots. The root specimen was attached to a Universal Test Machine (AGS-X, Shimadzu, Kyoto, Japan) for a push-out strength test, which was performed from coronal to apical in the universal testing machine. Size 30, 50 and 80 plungers were attached to testing machine for apical, middle and coronal segments, respectively; The loading speed was applied at 0.5 mm/min. The values were registered in Newtons for each specimen and then the force (F) was converted into tension (MPa) according to the following formula was used (19),  $MPa = F/Sealer\ adhesion\ area\ (SL)$  of each section was calculated as:  $SL = \pi \times (R + r) \times g$ ; r= apical radius, in mm; R = coronal radius, in mm;  $\pi = 3.14$ ; g = height relative to dentine cross section , in mm. One-way analysis of variance (ANOVA) and the Tukey test were used in analysis of data (P<0.05).

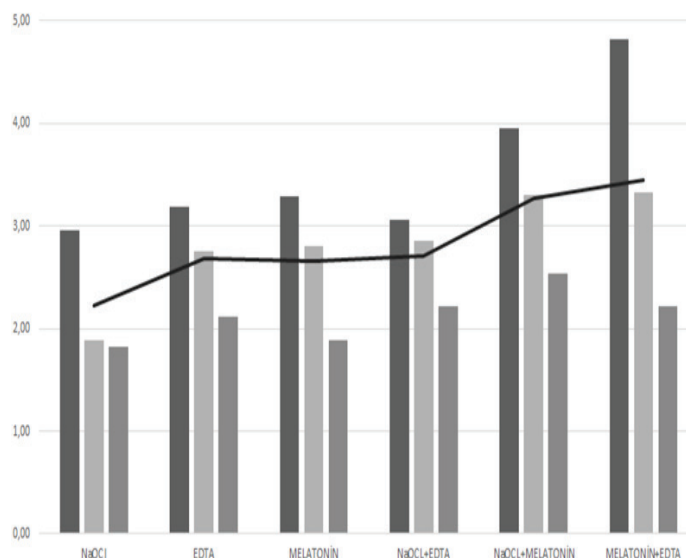
## RESULTS

Statistically significant differences between the irrigation protocols and the mean bond strength values were shown in Table 1. and Figure 1. In the coronal thirds, melatonin+EDTA group showed better bond strength than other groups (P<0.05), except for NaOCl+melatonin. In the middle thirds, NaOCl group showed significantly the lowest bond strength than other groups. (P<0.05). In the apical thirds, NaOCl and melatonin groups showed significantly worse bond strengths than other groups (P<0.05). In overall evaluation, push-out bond strengths of Melatonin+EDTA and NaOCl+Melatonin groups were significantly better than other groups (P<0.05) without no significant difference each other (P>0.05).

**Table 1. Evaluation of push-out bond strength values among groups in root canals**

Groups	Coronal	Middle	Apical	TOTAL
NaOCl	2.96±0.55 <sup>b</sup>	1.89±0.77 <sup>a</sup>	1.82±0.67 <sup>b</sup>	2.22±0.41 <sup>b</sup>
EDTA	3.19±0.22 <sup>b</sup>	2.75±0.55 <sup>b</sup>	2.11±0.23 <sup>a</sup>	2.68±0.32 <sup>b</sup>
Melatonin	3.29±0.59 <sup>b</sup>	2.80±0.39 <sup>b</sup>	1.89±0.34 <sup>b</sup>	2.66±0.29 <sup>b</sup>
NaOCl+EDTA	3.06±0.73 <sup>b</sup>	2.85±0.71 <sup>b</sup>	2.22±0.26 <sup>a</sup>	2.71±0.31 <sup>b</sup>
NaOCl+Melatonin	3.95±0.72 <sup>a</sup>	3.30±0.81 <sup>b</sup>	2.54±0.45 <sup>a</sup>	3.26±0.48 <sup>a</sup>
Melatonin+EDTA	4.82±0.8 <sup>4a</sup>	3.32±0.58 <sup>b</sup>	2.22±0.41 <sup>a</sup>	3.45±0.48 <sup>a</sup>

**Footnote: Values are expressed as mean ± SD in MPa. Different superscript letters in the same column indicate a significant difference among groups. (p < 0.05)**



**Figure 1. Bond strength mean values from root canals for each treatment**

## DISCUSSION

Since the irrigation solutions used during root canal treatment are in contact with the root dentin, the bonding of the canal filling material is affected (20-22). Unfortunately, previous studies reported that NaOCl-treated samples without the use of any chelating agent caused a negative effect on the bond strength of AH-Plus (23, 24). In many studies, Epigallocatechin-3-gallate, Ascorbic acid and Caffeic acid phenethyl ester are used because of their antioxidant properties (11, 25, 26). These suggested that it caused a change in dentine surface (11) and inhibits endogenous matrix metalloproteinases that cause hybrid-layer degradation (26) and affected the bond strength (11). The present study compared the effect of different root canal irrigations on bond strength of AH-Plus.

In terms of overall evaluation, the push-out bond strengths of Melatonin+EDTA and NaOCl+Melatonin groups were significantly superior to that of other groups. Therefore the null hypothesis was rejected.

NaOCl is both proteolytic agent which have the potential to denature the collagen components of the smear layer and a nonspecific oxidizing (27). NaOCl cause accentuated negative effect on the initiation of polymerization of the adhesive system, leading to lower bond strength (9). Similarly, among the materials used, NaOCl showed lower bond strength in the present study. The decreased bonding strength of NaOCl can be attributed to this reason.

EDTA, a well-known chelating agent, not only affects Ca ions, but can also cause demineralization areas in dentin (28). On the other hand, there is also study advocating that it does not cause erosion (29). EDTA is often suggested as an irrigant because can chelate, it remove the smear layer, prepare the dentinal walls for better adhesion of filling materials and be used the cleaning and shaping the canals with instruments (30). For these reasons, the push-out bond strength was evaluated for both solutions EDTA and Melatonin- an antioxidant property material, the results showed that the push-out bond strengths were statistically similar.

Melatonin has been reported to detoxify various free radicals and free oxygen radicals and is one of the most potent endogenous hydroxyl radical cleaners (28). Moreover, Melatonin has been shown to prevent from oxidative damage caused by free radical forming agents and events (31). In our study, the use of Melatonin after NaOCl solution showed high bond strength. We suggest that melatonin prevents the negative effects of NaOCl, it is attributed to the high antioxidant effect of melatonin.

It has been reported that when EDTA is used after NaOCl solution, collagen fibrils protect against the negative effect of NaOCl (29). Moreover, NaOCl-treated samples with the use of EDTA optimizes the bond strength of an epoxy resin sealer to dentine (24). In contrast, This study found no statistical difference between the final flush with EDTA and NaOCl+EDTA.

Push-out bond strength tests are used to measure the

tensile strength at the gutta-percha dentin interface. Push-out test results can be compared with clinical conditions. Goracci et al. reported that the push-out test was more reliable than conventional and modified microtensile tests for measuring bond strength (32). Therefore, in this study, push-out test was used to measure the strength of root canal filling materials.

## CONCLUSION

The bond strength of AH Plus was positively affected by Melatonin. Therefore, when using AH Plus as a root canal sealer, Melatonin could be preferred for irrigation agent. Further studies are needed to determine appropriate concentration and time used for melatonin.

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